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Multiple measures of HPA axis function in ultra high risk and first-episode schizophrenia patients

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Highlights

- Ultra-high risk and first-episode schizophrenia patients experienced more perceived stress and life events than the healthy controls
- Cortisol reactivity was increased in ultra high-risk patients compared to healthy controls and first-episode schizophrenia patients
- There was positive correlation between diurnal cortisol and severity of positive symptoms in the ultra-high risk group.
- There was no difference in pituitary gland volume between ultra-high risk and first-episode schizophrenia patients
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Abstract

Introduction

Abnormalities within hypothalamus-pituitary-adrenal (HPA) axis might interact with other neurobiological systems to enhance the risk of psychosis. Most of the neurodevelopmental and HPA axis changes occur in adolescence; this is also the period when prodromal and psychotic symptoms occur for the first time. More knowledge about how various stress components interact can advance understanding of the link between psychosis and the HPA axis.

Method

We examined 41 ultra high-risk (UHR) patients and 40 antipsychotic-naïve first-episode schizophrenia (FES) patients and compared them with 47 matched controls. The Perceived Stress Scale and the Recent Life Events Questionnaire were used to assess the stress levels. Day-time saliva samples were taken to measure cortisol. The pituitary gland volume was measured manually on the structural MRI using stereology.

Results

Only the UHR patients, had a higher cortisol increase just after awakening ($p=0.009$) compared to healthy controls. In UHR patients, we found a negative correlation between cortisol increase after awakening and symptom severity ($p=0.008$). Pituitary gland volume and diurnal cortisol were not

significantly different among the three groups. There was no correlation between pituitary gland volume, perceived stress/recent life events and any of the cortisol measures or symptoms.

Conclusion

Symptom severity during the very early phase of illness (UHR) seems to be associated with altered cortisol increase. Longitudinal studies in UHR patients would be useful to examine how stress levels affect the course of the illness.

Keywords: Ultra high risk; first-episode schizophrenia; HPA axis; cortisol; pituitary gland.

1. Introduction

For decades researchers have studied the hypothalamus-pituitary-adrenal (HPA) axis in psychosis, hypothesizing that hyperactivity in this axis reflects stress-related hormonal dysregulation (Walker et al., 2008). The extended neural diathesis-stress model (Pruessner et al., 2017) suggests a very complex interaction between vulnerability factors, neurodevelopmental alterations and psychosis progression. According to the model, genetic and environmental factors in early life increase the vulnerability for psychosis by promoting degenerative processes that affects neuromaturation. Early or later environmental insults contribute to dysregulation of the HPA axis, and disturbance in HPA axis function can enhance the psychotic symptoms. The most vulnerable period is the adolescence due to greater magnitude of the HPA axis response. The data supporting the hypothesis (Aiello et al., 2012; Borges et al., 2013) suggest that elevated cortisol may cause a sensitized dopaminergic response to stress, which can lead to excessive dopamine release (Mizrahi et al., 2012) that results in the onset of psychotic symptoms (Pruessner et al., 2017; Walker and Diforio, 1997).

In agreement with the afore mentioned diathesis-stress model, vulnerable individuals often develop psychosis – or psychosis-like symptoms and decline in functioning during adolescence where they first seek help. According to the criteria of being patients at ultra

high risk (UHR) of developing psychosis (Yung et al., 2005) some of these patients will develop psychosis or schizophrenia. For decades researchers have tried to identify patients who are in a prodromal phase of psychosis, and the UHR patients are thought to be in a prodromal phase. In a meta-analysis (Fusar-Poli et al., 2012a) of approximately 2500 high risk individuals, there was a mean transition rate of 36% (30% to 43 %) after three years. In individuals who will later transition to psychosis, most will develop a DSM/ICD schizophrenia spectrum disorder (Fusar-Poli et al., 2013). UHR patients, and adolescents with schizotypal personality disorder, present higher distress in response to life events and daily hassles compared to healthy controls (HCs) (Palmier-Claus et al., 2012; Pruessner et al., 2011; Tessner et al., 2011). Similarly, a recent meta-analysis found greater basal cortisol levels (morning) in UHR patients compared to HCs (Chaumette et al., 2015). Higher distress in response to stressors has also been observed in children at-risk for psychosis because they present a family history of illness or multiple antecedents (Cullen et al., 2014, British Journal of Psychiatry) which is important because these children are not medicated or help-seeking.

The cortisol awakening response (CAR), defined as the increase in cortisol released in response to waking up, has been studied as a potential biomarker in schizophrenia. A meta-analysis (Berger et al., 2016) showed that CAR is attenuated in patients with psychosis compared to HCs, and subgroup analysis showed flattened CAR in patients with schizophrenia and first-episode psychosis, but not in individuals with at-risk mental states.

In response to stress, the hypothalamus secretes corticotrophin releasing hormone, which stimulates adrenocorticotrophic hormone (ACTH) secretion from the anterior part of the pituitary gland. ACTH then stimulates the release of cortisol from the adrenal cortex.

Previous studies show that ACTH and cortisol are elevated during the early phases of psychosis (Mondelli et al., 2010; Pariante and Lightman, 2008) and that the pituitary gland volume increases before onset, as well as during the early stages of psychosis (Garner et al., 2005; Pariante et al.,

2005;Takahashi et al., 2009). An enlargement of the pituitary gland could be interpreted as an increase in number and size of corticotroph cells (Axelson et al., 1992;Pariante et al., 2004), and a large pituitary gland is thought to reflect a higher release of ACTH. This is supported by animal studies showing that the corticotroph pituitary cells increase in size and number when stimulated by corticotrophin releasing hormone (Westlund et al., 1985), as well as by evidence that UHR individuals who eventually developed psychosis had larger pituitary volumes compared to those who did not develop psychosis (Garner et al., 2005). The literature on pituitary volume abnormalities in UHR and first-episode schizophrenia (FES) patients is, however, very inconsistent, which we have previously demonstrated in a meta-analysis (Nordholm et al., 2013)

The findings on larger pituitary volume, increased levels of ACTH and cortisol suggest a disease model where HPA hyperactivity increases before the onset of psychotic symptoms and is pronounced during the first psychotic episode (Aiello et al., 2012;Borges et al., 2013).

To our knowledge, the present study is the first to investigate CAR, diurnal cortisol and pituitary gland volume, in addition to recent life events and perceived stress in UHR patients, antipsychotic-naïve FES patients and HCs. Our study provides a unique opportunity to examine how these markers are affected and associated with each other and with symptomatology in UHR and FES patients compared to HCs.

We hypothesized that cortisol levels during the day and pituitary volume would increase stepwise from HCs to UHR patients to FES patients, and thereby demonstrating a pattern of increasing alterations during the course of the illness. Additionally, we hypothesized that there would be an association between recent life events, perceived stress, cortisol levels and pituitary gland volume, which could confirm the connection between stress and psychosis, possibly leading to a better understanding of the importance of targeting stress levels in the early phases of psychosis.

2. Method

Conducted in accordance with the Declaration of Helsinki II the study was approved by the Regional Ethics Committee of the Capital Region of Denmark (FES: H-D-2008-088 and UHR: H-D-2009-013). All participants provided signed informed consent.

2.1. Ultra-high-risk patients

Forty-one UHR patients were recruited at the Mental Health Center Copenhagen, Denmark as part of a Danish prodromal project (see appendix). The diagnosis was based on a semi-structured interview using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). Additionally, we assessed symptoms in the UHR group with Comprehensive Assessment of At-Risk Mental States (CAARMS) (Yung et al., 2005), where symptom severity is the summed scores of the product of the global rating scale score (0-6) and the frequency score (0-6) of the four subscales of positive symptoms (Morrison et al., 2012). Two assessors were involved in determining whether or not to include a patient. Because Denmark does not have any UHR services/clinics, patients are treated in the psychiatric service based on their main UHR diagnosis, e.g., anxiety, depression, personality disorder or schizotypal disorder. When the clinicians suspected that a patient fulfilled the criteria for UHR status, they referred him, or her, to the researchunit. If the researcher considered a possible UHR status, they would do a CAARMS screening. Table 1 specifies the inclusion and exclusion criteria.

2.2. Patients with first-episode schizophrenia

Forty antipsychotic-naïve patients with FES were recruited, assessed and treated at the Mental Health Center Glostrup, Denmark. They were all a part of a large multimodal first-episode project called the Pan European Collaboration on Antipsychotic Naïve Schizophrenia (PECANS) project (see appendix). The patients underwent a comprehensive assessment battery including MRI and salivary cortisol, at baseline while they were antipsychotic naïve. The diagnosis was based on a semi-structured diagnostic interview using Schedules for Clinical Assessment in Neuropsychiatry (SCAN), version 2.1 (Wing et al., 1990). Psychopathology was measured using the Positive and

Negative Syndrome Scale, (PANSS) (Kay et al., 1987), while level of functioning was measured with the Social and Occupational Functioning Assessment Scale (SOFAS) (Goldman et al., 1992) by trained assessors. Duration of untreated illness was defined as the duration of psychotic symptoms with an impact on the level of functioning. Table 1 specifies the inclusion criteria.

2.3. Healthy controls

Forty-seven HCs were recruited from the community by advertising on a website for HCs to participate in studies. At baseline, they underwent a SCAN interview (Glostrup) or a SCID-I interview (Copenhagen), after which they were matched at group level for age and gender with the two patient groups.

FES patients, UHR patients and HCs were divided into three groups (A,B and C) based on parental socioeconomic status, where group A had the highest level of income and education and group C the lowest (Table 2).

2.4 Structural MRI

Structural brain images were acquired using a Phillips Achieva 3 Tesla whole-body magnetic resonance imaging (MRI) scanner (Phillips Healthcare, Best, The Netherlands) with an eight-channel SENSE Head Coil (Invivo, Orlando, Florida, USA) at Glostrup University Hospital, Denmark. Specifically, a high-resolution 3D T1-weighted imaging sequence was used (TR: 10.0ms; TE: 4.6 ms; TI 965.9 ms; flip angle 8 degrees). Acquiring 200 slices (sagittal plane) with a field of view 240 x 240 x 160 mm and a resolution matrix of 320 x 320 resulted in a final voxel size of 0.75 x 0.75 x 0.80 mm.

Total brain tissue volume was estimated with SIENAX (Smith et al., 2002), part of FSL (Smith et al., 2004), beginning with the extraction of brain images from the individual whole-head input data and followed by tissue-type segmentation with partial volume estimation (Zhang et al., 2001).

2.5 The pituitary gland volume

Each pituitary was measured by the same investigator (DN) using stereology in the computer program MEASURE (version 0.8, Johns Hopkins University, Baltimore, MD), the details of which have been previously reported (Schulze et al., 2003).

The investigator was blind to group status and subject identification. A 3D grid with spacing of one voxel was superimposed on the MRI images. Volume of the pituitary gland was automatically calculated in cubic millimeters by summing volumes for all relevant slices.

The pituitary stalk was excluded and the posterior pituitary gland was included, as were the usually well-defined borders of the anterior and posterior pituitary: diaphragma sellae, superiorly; the sphenoid sinus, inferiorly; and the cavernous sinuses, bilaterally. Based on 10 reference images, the intraclass correlation coefficient was >0.90 and the intrarater reliability was 0.98.

2.6 Stress scales

The Perceived Stress Scale (PSS) (Cohen et al., 1983) has previously been validated in Danish populations (Nielsen et al., 2008). Its purpose was to measure the degree to which life situations were appraised as stressful (past two weeks) by considering coping resources and feelings of control (Cohen et al., 1983). The scale consists of 10 questions, with a total score ranging from zero to 40 (high scores = high levels of perceived stress).

We collected information about recent life events (past six months) using the Brief Life Events Questionnaire (Brugha and Cragg, 1990). The questionnaire assesses the number of negative life events, such as illness or injury, death of a close friend or relative, unemployment, financial loss and loss of important relationships.

2.7 Salivary cortisol

Saliva samples were collected by absorption into Visispear sponges (Beaver-Visitec), which were stored in Salivette tubes (Sarstedt, Leicester, UK). Subjects were instructed to collect saliva by putting two Visispears under the tongue and doing chewing movements for at least two minutes. Saliva was collected immediately after awakening, and 15, 30 and 60 minutes after awakening (before 10 am). Participants were instructed not to drink, eat, smoke or brush their teeth for 30

minutes before taking a sample. They repeated the sample collection at 12 pm and 8 pm. Subjects were told to keep the samples in the refrigerator until they were sent for analysis, within 72 hours. The tubes were centrifuged at 4500 RPM for 10 minutes, the Visispears removed and the saliva frozen at minus 20°C until analysis. Cortisol levels were determined using the High Sensitivity Salivary Cortisol ELISA KIT (Salimetrics) following the previously described procedure (Mondelli et al., 2015). Optical density was read at 450 nm, with correction at 620 nm using a Beckman Coulter DTX 880 plate reader with Multimode Detection Software 2.0.0.12. The data were analyzed using SoftMax Pro 4.8.

2.8 Statistics

The data were checked for normality of distribution, the mean values between the three groups were analyzed by using one-way analysis of variance (ANOVA) with the Tukey post hoc test. When comparing only two groups (e.g., males vs. females) we used an independent t-test. Pearson's chi-squared test was used for categorical data. Person's correlation coefficient was used to test the correlation between two variables.

2.8.1 Statistics and cortisol

Diurnal cortisol was based on three cortisol samples (at time of awakening ($t=0$), 12 pm and 8 pm) as area under the curve with respect to the ground (AUC_g) using the previously described trapezoidal method (Pruessner et al., 2003). CAR was presented as area under the curve with respect to increase (AUC_i) according to new consensus guidelines (Stalder et al., 2016) using the previously described trapezoidal method (Pruessner et al., 2003) and considered cortisol levels at $t=0$ and 15, 30 and 60 minutes after awakening. AUC_i was calculated as the AUC_g after subtraction of the value at $t=0$ (Pruessner et al., 2003).

Cortisol reactivity was defined as the cortisol increase from $t=0$ to the first measurement after awakening ($t=15$). Cortisol recovery was defined as the cortisol at 8 pm subtracted from the highest cortisol level during CAR.

2.8.2 Missing samples of saliva

If only one sample of saliva was missing from a patient/control, the value was replaced by the mean of the two adjacent values; if the missing value was either at awakening or 8 pm, we replaced the value with the mean value of all participants. If one subject had more than one missing sample, we did not compute the subject's AUC. Other researchers have previously used this method of handling missing data (Jorgensen et al., 2013).

Data were analyzed using the Statistical Package for Social Sciences, Version 20.0 (SPSS Inc.).

3. Results

3.1 Patients and controls

Level of functioning (SOFAS) was similar in the UHR ($M=43.1$, standard deviation (SD) 6.4) and FES patients (43.4 , $SD=11.0$, $t(75)=-0.113$, $p=0.910$) (Table 2). The groups did not significantly differ on age, sex, height or weight or level of functioning, but there was group differences in education (table 2) ($F(2,115)=11.8$, $p<0.001$), where UHR ($M=12.9$, $SD=2.9$, $p<0.001$) and FES ($M=12.2$, $SD=2.3$, $p=0.001$) patients attended school for a shorter time compared to healthy controls ($M=15.0$, $SD=2.7$).

Of the 41 UHR patients, 39 underwent an MRI scan and 32 provided saliva. Of the 40 FES patients, 39 underwent an MRI scan and 26 provided saliva. Reasons for not undergoing an MRI scan were exclusion due to dental brace, refusal to be scanned, or dropping out of the study before completing the baseline examinations. Reasons for not providing saliva were use of hormonal contraception, refusal to provide saliva, or difficulties in undertaking the procedure.

For UHR patients, seven received antipsychotics and were minimally treated (Table 1).

The mean DUI for FES patients was 61.8 weeks ($SD=63.0$) and the median was 36 weeks (range 2 to 300 weeks).

None of the UHR and FES patients, or the controls, had a current abuse/dependency of either alcohol or illicit drugs.

There was a significant difference among the three groups regarding parental socioeconomic status (Pearson's chi-squared test), χ^2 (df: 4, N=121)=15.26, $p=0.004$ (Table 2). None of the UHR patients' parents were in socioeconomic group C, compared with 22.9% of FES patients and 15.6% of HCs (Table 2).

3.2 Cortisol

Eighty-six subjects (28 HCs, 32 UHR patients and 26 FES patients) completed salivary cortisol collection. Two UHR patients, four FES patient and three healthy controls had one missing sample of saliva, where data was imputed as described in the section of statistics.

There were differences between groups (one-way ANOVA) in cortisol reactivity (the cortisol increase from $t=0$ to the first measurement after awakening ($t=15$)), ($F(2, 84)=4.94$, $p=0.009$). Post hoc comparisons using a Tukey post hoc test revealed that the cortisol reactivity was significantly higher in UHR patients ($M=4.36$, $SD=4.22$ nmol/l, $p=0.008$) compared with HCs ($M=1.58$, $SD= 2.91$ nmol/l). There was no statistical difference between cortisol reactivity in HC ($p=0.530$) compared with FES ($M=2.59$, $SD=3.02$ nmol/l) or between cortisol reactivity in UHR and FES ($p=0.132$).

There was no significant effect of group status on either CAR, diurnal cortisol or cortisol recovery (the cortisol at 8 pm subtracted from the highest cortisol level during CAR).

(Table 3). Figure 1 shows the diurnal variation.

We further examined whether the minimal use of antipsychotics in UHR patients had any impact on cortisol. There were no differences (two-sample t-test) between cortisol reactivity, CAR or diurnal cortisol, in UHR patients who were treated with low dosage antipsychotics versus antipsychotic-naïve UHR patients ($p>0.05$).

3.3 Pituitary gland volume

One-hundred and twenty subjects (43 HCs, 39 UHR patients and 38 FES patients) underwent MRI scans. There was no difference in pituitary gland volume among the three groups (one-way ANOVA) ($F(2, 117)=1.04$, $p=0.358$) (Table 3).

Across the UHR sample (two-sample t-test) there was no difference in pituitary volume in patients who were treated with a small dosage of antipsychotics ($M=0.692$, $SD=0.149$ cm³) and antipsychotic-naïve patients ($M=0.764$, $SD=0.188$), ($p=0.383$).

Pituitary volume (two-sample t-test) in males ($M=0.79$, $SD=0.17$) were larger compared to females ($M=0.72$, $SD=0.14$), ($p=0.053$), but it not reach statistical significance.

3.4 Perceived stress and recent life events

There were significant differences between HCs, UHR patients and FES patients (one-way ANOVA) in perceived stress ($F(2, 93)=73.35$, $p<0.001$) and recent life events ($F(2, 94)=5.80$, $p=0.004$) (Table 2). Post hoc comparisons using a Tukey post hoc test showed that UHR ($M=25.12$, $SD=6.48$ point, $p<0.001$) and FES patients ($M=24.62$, $SD=5.31$ points, $p<0.001$) experienced significantly more perceived stress compared with HCs ($M=10.03$, $SD=5.03$ points). There was no statistically significant difference between perceived stress in UHR and FES patients ($p=0.939$).

FES patients ($M=2.04$, $SD=2.29$, $p=0.003$) experienced significantly more recent life events compared with HCs ($M=0.76$, $SD=0.75$). There was no difference between UHR ($M=1.35$, $SD=1.0$, $p=0.178$) and HCs, or between UHR and FES patients ($p=0.143$).

3.5 Association between stress scales, cortisol and pituitary gland volume

To investigate the relationship between perceived stress, life events, cortisol and pituitary volume in all the patients and controls, Pearson's correlation coefficient (ρ) was computed. There was a positive correlation between perceived stress and life events ($\rho=0.314$, $n=95$, $p=0.002$). None of the other measures were positively correlated ($p>0.06$).

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3.6 Association between symptom severity, stress scales, pituitary volume and cortisol.

We also computed Pearson's correlation coefficient to investigate the relationship between the measures of stress and symptom severity (see method section 2.1). Symptom severity is the

summed score of the product of the global rating scale score (0-6) and the frequency score (0-6) of the four subscales of positive symptoms. In UHR patients, we found a negative correlation between the CAARMS symptom ($p = -0.471$, $n = 31$, $p = 0.008$). Our study did not find any significant correlations ($p > 0.06$) between perceived stress/life events, CAR, diurnal cortisol, pituitary gland and CAARMS symptom severity or total PANSS positive.

Within-group correlations were not different to the results obtained across the total sample.

4. Discussion

This is the first study to report on cortisol increase, CAR, diurnal cortisol, stress scales and pituitary volume in antipsychotic-naïve FES patients and minimally treated UHR patients. The UHR patients, but not the FES patients, had significantly higher cortisol reactivity compared with HC. There was no significant difference in CAR and diurnal cortisol between the three groups. We found a negative correlation between CAARMS symptom severity and cortisol reactivity in UHR patients. Perceived stress and recent life events were correlated. Perceived stress was significantly higher in both UHR and FES patients compared to HCs, and recent life events were significantly higher in FES patients compared to HCs. Pituitary gland volume did not vary significantly between the three groups of subjects and it was not correlated with any symptoms, cortisol or stress scales.

4.1 Cortisol

The magnitude of CAR and the diurnal cortisol level seem to be indicators of HPA axis activation. Mildly stressed individuals, e.g., due to work or general life conditions, seem to have increased CAR_i (Chida and Steptoe, 2009; De et al., 2003; Kunz-Ebrecht et al., 2004) or cortisol reactivity (Kunz-Ebrecht et al., 2004). People who suffer from chronic and severe conditions, e.g., burnout, exhaustion or posttraumatic stress syndrome present a blunted awakening response (Chida and Steptoe, 2009) and people who suffer from, e.g., psychosis and major depression, seem to have a higher level of diurnal cortisol (O'Brien et al., 2004; Walker et al., 2008).

The UHR patients in our study had increased cortisol reactivity similar to that of mildly stressed individuals (Chida and Steptoe, 2009; De et al., 2003; Kunz-Ebrecht et al., 2004). Cortisol reactivity was negatively correlated with the positive symptoms in UHR patients. Day et al. (Day et al., 2014) found blunted CAR in UHR patients compared with HCs but they were unable to show any correlation between symptoms and CAR. One of the reasons could be the use of antipsychotics and the fact that most patients received therapy, while our patients had recently been included in treatment and most were antipsychotic-naïve. Furthermore, the cohort from the paper by Day et al was referred directly to an established prodromal service in southern London due to the UHR symptomatology, mostly because of attenuated positive symptoms. Our patients were identified by the clinicians in mental health centers and referred to the research unit where they were invited to participate in a screening. Accordingly, the UHR groups might not be directly comparable.

4.1.1 Cortisol reactivity, CAR and symptom severity

Our data shows that cortisol reactivity is higher in UHR patients and appears to decrease when the intensity and frequency of positive symptoms increase. One of the reasons for this variance could be that we examined the patients at an early stage, where CAR is still progressively being blunted. On the other hand, we were not able to show the blunted CAR in the FES patients. When we compare the three groups with previous studies, our control group has a lower cortisol response to awakening at 15 and 30 minutes (Day et al., 2014; Mondelli et al., 2010). The low awakening response in HCs might lead to an overestimation of cortisol response in UHR and FES patients and thus fail to show the expected blunted response in UHR and FES patients. The lack of blunted CAR in FES/schizophrenia patients is in agreement with previous findings (Hempel et al., 2010; Pruessner et al., 2013) and the blunted response might not be fully explained by the acute psychotic symptoms.

The negative correlation between the total CAARMS score and cortisol reactivity could be due to stress and attenuated psychotic symptoms that are associated during the early phase of illness, before the onset of full blown psychosis. One possible explanation is that increased cortisol stimulates dopamine activity (Mizrahi et al., 2012; Walker and Diforio, 1997) and consequently

psychotic symptoms in UHR patients, whereas the increased dopamine activity repeatedly found to be associated with psychosis in the schizophrenia patients is independent of cortisol levels.

A meta-analysis (Chaumette et al., 2015) of salivary cortisol in early psychosis describes other reasons for the lack of difference between FES patients and HCs. For example, cortisol abnormalities are not specific to psychosis, but the distress associated with emerging illness may be the reason for an increased level of cortisol (in our study, cortisol reactivity), rather than a specific biomarker of psychosis. Another study highlights the importance of comorbidities, adherence, response to medication and objective verification of sampling adherence (Berger et al., 2016). In future studies it would be of great interest to implement an objective verification, e.g. by monitoring the activity of the patient.

4.1.2 Hypotheses about CAR

It has been hypothesized that CAR represents a stress response to the anticipated demands of the forthcoming day (Fries et al., 2009), and a blunted response has been interpreted as impaired responsiveness to increased stress (Aiello et al., 2012). Recent expert consensus guidelines, however, describe that deviations from a typical CAR pattern are assumed to mark maladaptive neuroendocrine processes (Stalder et al., 2016) and that researchers need to be aware of not mistaking CAR for a general HPA activity marker. Likewise, CAR is unrelated to cortisol reactivity to experimentally-induced psychological stress (Stalder et al., 2016). It is important to note the large variability of cortisol levels and other pathophysiological mechanisms, as several biological factors could affect cortisol levels. Moreover some subgroups of patients might be more stress sensitive than others or have a dysfunctional stress response.

4.2 Pituitary gland

We did not find any differences in pituitary gland volume between UHR patients, FES patients and HCs. The literature contains studies with conflicting results, as a meta-analysis we previously conducted demonstrated (Nordholm et al., 2013). One possible explanation for the inconsistent results is the fact that the duration of illness varies greatly and the studies are small (Nordholm et

al., 2013). Another potential reason is that psychotic disorders are complex with regard to clinical symptoms, as well as pathogenesis and pathophysiology, which is why enhanced pituitary volume in UHR and FES patients could be related to a subgroup of patients. Some studies have reported differences between converters and non-converters in an UHR sample (Buschlen et al., 2010; Garner et al., 2005) whereas other studies were unable to replicate these findings (Gruner et al., 2012; Walter et al., 2015). Finally, measuring the pituitary volume provides information about changes in the corticotroph cells, which is important since the anterior pituitary gland also contains a variety of other cell types. Anterior/posterior gland subdivision is not possible in this technique of manual tracing.

4.3 Stress scales

The higher levels of perceived stress in patients compared with controls were expected. The HC scores were representative, since they matched the scores from large samples of the Danish population (Nielsen et al., 2008). The lack of difference in perceived stress between UHR and FES patients was unexpected, but the literature suggests that a low level of education, lack of social network and poor working conditions are associated with perceived stress (Nielsen et al., 2008). These conditions are also present in UHR and FES patients, both of which had a low level of functioning. It is also important to remember that anxiety and worrying are common comorbidities in the high-risk population (Fusar-Poli et al., 2012b; Rosen et al., 2006), leading to the increased risk of high perceived-stress scores in the UHR population. In some studies the UHR patients even exceed the perceived stress ratings in the FES patients (Palmier-Claus et al., 2012; Pruessner et al., 2011).

Recent life events were significantly higher in FES patients, but not UHR patients, compared to HCs. We did not find a significant difference, however, in the number of recent life events between UHR and FES patients. As expected, though, the recent life event score of UHR patients was in between that of HCs and FES patients, but current or recent life event might be of less importance

than childhood trauma (Varese et al., 2012), which has been strongly linked to increased risk of developing psychosis.

4.3.1 Stress scales and cortisol

The lack of correlation between perceived stress and recent life events versus CAR (AUCi) in schizophrenia, FES and UHR patients is supported by previous findings (Jorgensen et al., 2013; Thompson et al., 2007), though some studies report an association between an increased number of stressors and a blunted CAR (Brenner et al., 2009; Wong et al., 2012). As previously described, life events might enhance the risk of developing psychosis and studies show that there is a significant association between the two (Bak et al., 2005; Rubino et al., 2009), but the direction of causality cannot be inferred and the high levels of perceived stress and life events may partially be due to recall bias. A systematic review and meta-analysis (Beards et al., 2013) of adult life events and psychosis onset was only able to identify 16 studies. The authors concluded that there are some indications that intrusive events may be relevant to the development of psychosis, but much of the existing research is methodologically limited. This supports our findings of a lack of correlation between life events, cortisol and psychotic symptoms. In future studies, it would be interesting to investigate daily hassles and accumulated life events since the life events in this study only included ones that occurred within the past six months, leading to an underestimation of the importance of the events.

4.4 pituitary gland and cortisol

Our data show no association between cortisol and pituitary volume, which could be explained by a modest HPA axis hyperactivity. Thompson et al (Thompson et al., 2007) also illustrated lack of association between cortisol/pituitary volume and symptomatology, and they suggested that the number of hassles were of greater importance than symptomatology.

4.5 Study limitations

We did not measure quality of sleep, hours of exercise/activity, preferred time of awakening or hours of exposure to daylight, which are potential confounders of the results. Accumulated minor

daily hassles were not reported and could also contribute to an increased stressful state. It is also important to note that due to small sample we are aware of a risk of statistical type-2 errors.

In future studies it would be interesting to follow UHR patients until they become psychotic and thereby examine the different stages from UHR to FES in a longitudinal design. Unfortunately, we did not have data on depression and anxiety and therefore cannot exclude that these affective symptoms could be associated with the CAR and diurnal cortisol response seen in the UHR group.

4.6 Conclusion

Our data suggest that UHR patients show a minor pattern of maladaptive neuroendocrine processes with increased cortisol reactivity, which our findings show was not the case for the FES patients. We found a negative correlation between symptoms in UHR patients and cortisol reactivity. This may mean that some UHR patients are sensitive to stress during the early course of the illness.

Together with previous studies, the present data on stress and psychosis, contribute with important knowledge on the mechanisms of stress that might lead to deterioration of psychosis. In research and clinical settings it would be useful to be able to identify these vulnerable patients in order to prevent deterioration.

Longitudinal studies in UHR patients would be useful to examine how the course of illness correlates with the level of stress.

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Appendix: Publication list for our research groups: a) ultra-high-risk patients and b) first-episode schizophrenia patients

a)

Dannevang AL, Randers L, Gondan M, Krakauer K, Nordholm D, Nordentoft M, 2016: Premorbid adjustment in individuals at ultra-high risk for developing psychosis: a case-control study. *Early Interv Psychiatry*;10.

Krakauer K, Nordentoft M, Glenthøj BY, Raghava JM, Nordholm D, Randers L, Glenthøj LB, Ebdrup BH, Rostrup E, 2018: White matter maturation during 12 months in individuals at ultra-high-risk for psychosis. *Acta Psychiatr Scand*;137(1):65-78.

Krakauer k, Ebdrup BH, Glenthøj BY, Raghava JM, Nordholm D, Randers L, Rostrup E, Nordentoft M, 2017: Patterns of white matter microstructure in individuals at ultra-high-risk for psychosis: associations to level of functioning and clinical symptoms. *Psychol Med*; 47(15):2689-2707.

Madsen HK, Nordholm D, Krakauer K, Randers L, Nordentoft M, 2017: Psychopathology and social functioning of 42 subjects from a Danish ultra high-risk cohort. *Early Interv Psychiatry* 19.

Nordholm D, Poulsen HE, Hjorthøj C, Randers L, Nielsen MO, Wulff S, et al., 2016: Systemic oxidative DNA and RNA damage are not increased during early phases of psychosis: A case control study. *Psychiatry Res* 241:201-206.

b)

Bak N, Ebdrup BH, Oranje B, Fagerlund B, Jensen MH, During SW, et al., 2017: Two subgroups of antipsychotic-naïve, first-episode schizophrenia patients identified with a Gaussian mixture model on cognition and electrophysiology. *Transl Psychiatry* 7:e1087.

During S, Glenthøj BY, Andersen GS, Oranje B, 2014: Effects of dopamine D2/D3 blockade on human sensory and sensorimotor gating in initially antipsychotic-naïve, first-episode schizophrenia patients. *Neuropsychopharmacology* 39:3000-3008.

During S, Glenthøj BY, Oranje B, 2015: Effects of blocking D2/D3 receptors on mismatch negativity and P3a amplitude of initially antipsychotic naïve, first episode schizophrenia patients. *Int J Neuropsychopharmacol* .

Ebdrup BH, Raghava JM, Nielsen MO, Rostrup E, Glenthøj B, 2015: Frontal fasciculi and psychotic symptoms in antipsychotic-naïve patients with schizophrenia before and after 6 weeks of selective dopamine D2/3 receptor blockade. *J Psychiatry Neurosci* 41:150030.

Nielsen MØ, Rostrup E, Broberg BV, Wulff S, Glenthøj B, 2018: Negative symptoms and reward disturbances in schizophrenia before and after antipsychotic monotherapy. *Clin EEG Neurosci*. Jan;49(1):36-45.

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Nielsen MO, Rostrup E, Wulff S, Glenthøj B, Ebdrup BH, 2016: Striatal reward activity and antipsychotic-associated weight change in patients with schizophrenia undergoing initial treatment. *JAMA Psychiatry*. 73(2):121-8

Nørbak-Emig H, Pinborg LH, Raghava JM, Svarer C, Baaré WFC, Allerup P, Friberg L, Rostrup E, Glenthøj B, Ebdrup BH, 2017: Extrastriatal dopamine D_{2/3} receptors and cortical grey matter volumes in antipsychotic-naïve schizophrenia patients before and after initial antipsychotic treatment. *World J Biol Psychiatry*. Oct;18(7):539-549.

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Table 1. Inclusion and exclusion criteria for patients with first-episode schizophrenia, patients at ultra high risk of developing psychosis and healthy controls

Healthy controls	First-episode psychosis	Ultra high risk of psychosis
Inclusion criteria		
18 to 45 years old	18 to 45 years old Diagnosis of schizophrenia or schizoaffective psychosis (ICD-10) ^{a)}	18 to 40 years old Fulfill CAARMS ^{b)} criteria for at least one of three at-risk groups: <ul style="list-style-type: none"> - Attenuated positive symptoms - Brief limited, intermittent psychotic symptoms - Trait and state of risk factor As well as a decline in functioning (at least a 30% drop in SOFAS score, sustained for at least one month), or sustained low functioning (SOFAS score ≤ 50 for at least one year)
Exclusion criteria		
Any previous or current psychiatric illness or drug abuse Any first-degree relatives with psychiatric diagnosis	Treatment with antipsychotics (lifetime) Use of antidepressants within the last month Current drug dependency (ICD-10) Treatment with methylphenidate (lifetime) History of major head injury	Past history of treated or untreated psychotic episode of \geq one week's duration Organic brain disease, e.g., epilepsy, inflammatory brain disease Any physical illness with psychotropic effect, if not stabilized Current treatment with mood stabilizers or methylphenidate Past antipsychotic exposure equipotent to a total lifetime haloperidol dose of >50 mg Diagnosis of a serious developmental disorder, e.g., Asperger's syndrome IQ <70 and a documented history of developmental delay or intellectual disability Current pregnancy Symptoms entirely explained by drug use
Patients with contraindications for MRI, including dental braces were excluded from the MRI. Patients using hormonal contraception were excluded from the cortisol measurement because of the risk of false-positive high cortisol concentrations.		

^{a)}ICD-10: International Classification of Diseases, Tenth Revision;^{b)}CAARMS: Comprehensive Assessment of At-Risk Mental States**Table 2.** Demographics, psychopathology and stress scales in healthy controls, patients at ultra high risk of developing psychosis and patients with first-episode schizophrenia (ANOVA).

HC/UHR/FES	Healthy controls (HCs) Mean +/- (SD)	Ultra-high-risk patients (UHR) Mean (SD)	First-episode schizophrenia patients (FES) Mean (SD)	P-value
Age (years) (N=46/41/40)	24.7 (5.5)	23.9 (4.71)	24.1 (4.8)	p=0.711
Gender male/total (% males)	28/48 58.3%	18/42 42.9%	22/40 55.0%	p=0.314
Height (cm) (N=19/41/38)	176.3 (9.0)	173.9 (9.3)	175.0 (10.0)	p=0.655
Weight (kg) (N=23/41/40)	70.9 (11.0)	76.0 (19.5)	74.8 (17.8)	p=0.736
Years of schooling/education (N=40/42/36)	14.9 (2.8)	12.9 ** (2.9)	12.2 ** (2.3)	p<0.001
Functioning (SOFAS score ^{a)})	-	43.1 (6.4)	43.4 (11.0)	p=0.910
Parental socioeconomic status A/B/C ^{b)}	17/20/7	23/17/0	8/19/8	p=0.006
Total brain volume (mm ³) N=43/39/35	1141.01 (100.15)	1138.78 (125.15)	1109.44 (92.29)	p=0.371
CAARMS ^{c)} scores (N=41)				
Unusual thought content, intensity		2.85 (1.49)	-	-
Unusual thought content, frequency	-	3.55 (1.78)	-	-
Non-bizarre ideas, intensity	-	3.54 (1.27)	-	-
Non-bizarre ideas, frequency	-	4.35 (1.31)	-	-
Perceptual abnormalities, intensity	-	2.85 (1.15)	-	-
Perceptual abnormalities, frequency	-	3.39 (1.50)	-	-
Disorganized speech, intensity	-	2.3 (0.86)	-	-
Disorganized speech, frequency	-	4.05 (1.83)	-	-
CAARMS symptom severity (total intensity + total frequency)	-	27.29 (4.88)	-	-
CAARMS subgroups (N=41)				
Trait and state	-	25	-	-
Attenuated positive symptoms	-	38	-	-
Brief limited, intermittent psychotic symptoms	-	3	-	-
PANSS ^{d)} (N=39)				
Positive	-	-	19.6 (3.4)	-
Negative	-	-	20.1 (7.5)	-
General	-	-	41.4 (7.9)	-
Total	-	-	81.1 (14.9)	-
Stress scales				
Perceived stress N=33/39/24	10.03 (5.03)	25.13* (6.48)	24.63* (5.31)	p<0.001
Recent life events (6 months) N=33/40/24	0.76 (0.75)	1.35 (1.10)	2.04** (2.29)	p=0.004

*) p<0,05 versus HCs;

**) p<0.01 vs. HCs;

a) SOFAS: Social and Occupational Functioning Assessment Scale;

b) Parental socioeconomic status was categorized into three groups based on income and education, with A as the highest and C the lowest;

c) CAARMS: Comprehensive Assessment of At-Risk Mental States;

d) PANSS: Positive and Negative Syndrome Scale.

Figure 1. Cortisol curves of the mean salivary diurnal cortisol (nmol/l) in healthy controls (Healthy), ultra-high-risk (UHR) patients and first-episode schizophrenia (FES) patients (including 95% confidence limits).

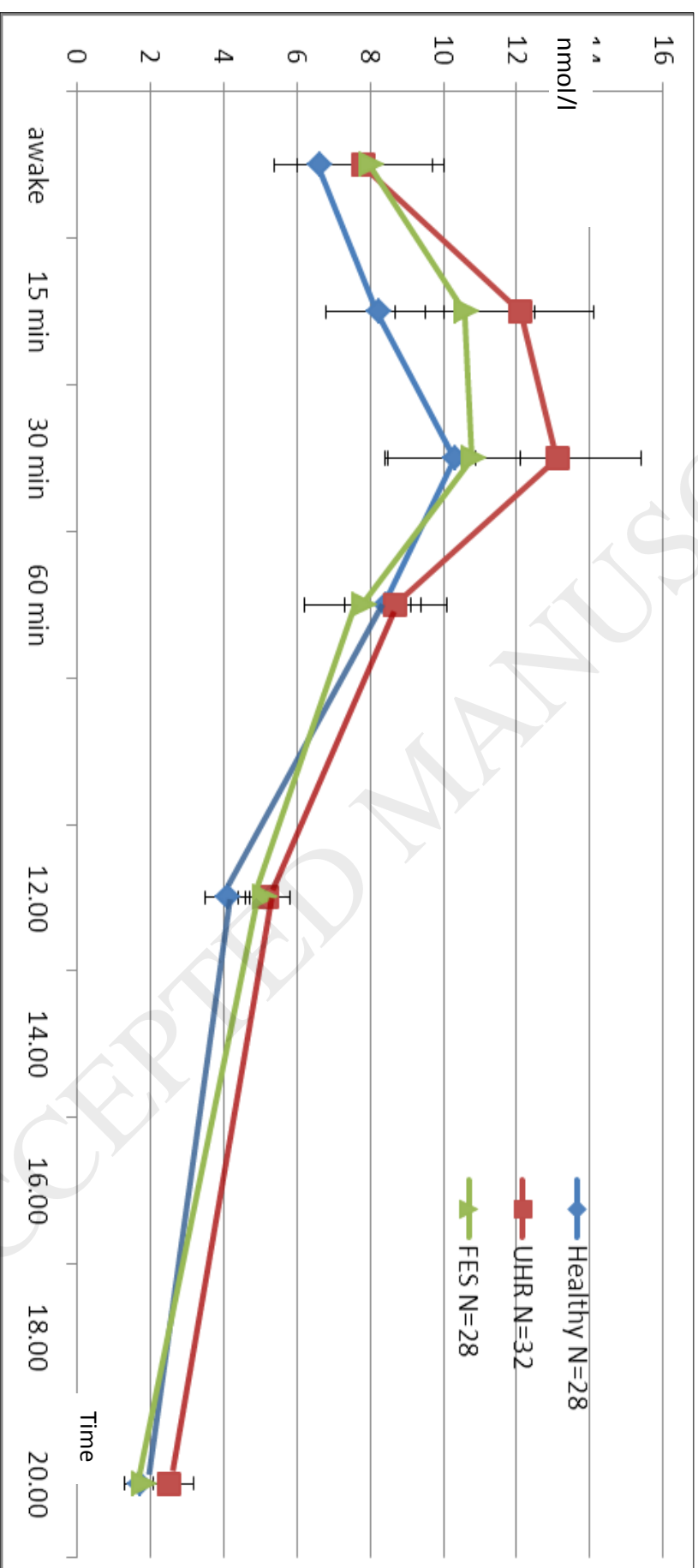


Table 3. Analysis of the cortisol levels and pituitary gland volume in healthy controls, ultra-high risk patients and antipsychotic naïve first-episode schizophrenia patients.

	Healthy controls Mean ^{*)} (CI) N=28	Ultra High risk Mean ^{*)} (CI) N=32	First-episode schizophrenia Mean ^{*)} (CI) N=26	F Value	P (overall) P<0.05
CAR ^{a)} (AUC ^{b)}) nmol min/l	158.50 (70.60 to 246.40)	199.19 (105.07 to 203.32)	99.93(16.27 to 183.60)	1.30	0.277
Diurnal cortisol ^{c)} (AUC ^{b)}) nmol min/l	2706.79 (2366.61 to 3046.98)	3435.84 (2850.32 to 4021.37)	3218.35 (2720.34 to 3716.37)	2.38	0.099
Cortisol reactivity ^{d)} nmol min/l	1.58 (0.45 to 2.70)	4.36** (2.83 to 5.89)	2.59 (1.39 to 3.79)	4.94	0.009***
Cortisol recovery ^{e)} nmol min/l	9.5 (7.73 to 11.33)	12.3 (10.14 to 14.45)	10.5 (8.25 to 12.70)	1.97	0.146
Pituitary gland volume mm ³ N= 43/39/39	0.73 (0.69-0.77)	0.75 (0.69 to 0.81)	0.78 (0.72 to 0.84)	1.04	0.358

*) ANOVA was used to calculate mean-values;

**) p<0.05;

***) p<0.01;

a) CAR: cortisol awakening response, cortisol at awakening and +15, +30 and +60 minutes after awakening;

b) AUC_i: Area under the curve with respect to increase and AUC_g: area under the curve with respect to the ground;

c) Diurnal cortisol: cortisol during the entire day, three measurements: at awakening, noon and 8 pm;

d) Cortisol reactivity: cortisol at +15 minutes minus awakening cortisol;

e) Cortisol recovery: the highest cortisol concentration in the morning minus the 8 pm concentration.